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Anticlastogenic and antioxidant activity of *Cassia fistula* against Cyclophosphamide induced micronucleus formation in Swiss albino mice

Maya Kushwaha[1] and Ramesh C Agrawal[2]
1 Dept of Bioscience, Barkatullah University, Bhopal
2 Department of Research, Priyamvada Birla Cancer Research Institute, Birla Hospital
kushwahamaya21@gmail.com, rcagrawal60@yahoo.com

ABSTRACT
Traditional Systems of medicines play an important role in global health care needs. More than 30% of the entire plant species are used for medicinal purposes. **Purpose:** The present investigation was undertaken to investigate the Anticlastogenic effect and antioxidant property of *Cassia fistula* (CF) extract against Cyclophosphamide (CP) induced micronucleus formation in the mouse bone marrow cells. **Methods:** The bone marrow micronucleus assay was undertaken for anticlastogenic activity However the fenton reaction method was used to study the antioxidant activity . **Results:** The single IP administration of CF seed extract at the dose of 500, 1000 and 1500 mg/kg body weight, 24 hours prior the administration of CP (at the dose of 50 mg/kg) have significantly prevented the micronucleus formations in dose dependent manner in bone marrow cells of mice as compared to CP group. In another experiment an in vitro antioxidant assay which is based on the oxidative damage to 2-deoxy-D-ribose induced by hydroxyl radicals (•OH) generated by the reaction between ascorbic acid and (Fe III)-EDTA has been examined. **Conclusion** : Our results suggest that *Cassia fistula* have a preventive potential against CP-induced micronucleus formation in *Swiss* mouse bone marrow cells probably due to its antioxidant properties.
Keywords: *Cassia fistula*, Mutagenicity, Micronucleus, Bone marrow, Cyclophosphamide.

INTRODUCTION

Medicinal plants are being used for the treatment of various diseases in different part of the world from time immemorial. Natural product from plant, bacteria, fungi and other organism continue to be used in pharmaceutical preparation either as pure compound or as an extract [1]. *Cassia fistula* Linn (Hindi-Amaltas) is a median size tree belonging to family Leguminosae is widely cultivated throughout India. It is widely used for its medicinal purposes. The main property of the plant is reported as a mild laxative[2],cure skin diseases[3], wound healing[4], Hypoglycemic[5], Antibacterial[6], Antifungal[7], Hypocholestremic[8], Hepatoprotective[1,9], Antitumour[10], Antioxidant[11,12], Antifertility[13] have been already investigated. In spite of vast pharmacological activity of this plant, its protective effect on mutagenicity has not been studied. We have therefore undertaken to study the antimutagenicity of plant extract using micronucleus in mice bone marrow cell and antioxidant activity using De oxy ribose method

MATERIALS AND METHODS

Collection & Identification of Plant

The pods of *C. fistula* were collected from local areas of Bhopal, M.P. (India). Authenticated by the Botanist Dr. Z. Hasan, Department of Botany, Safia Science College, Bhopal, Madhya Pradesh (India), where the herbarium was deposited. Voucher Specimen No: 294/bio/Safia/ 2011.

Project Approval: Project approved by Institutional Animal Ethical Committee (IAEC), Project no. 1695/PO/C/B/CPCSEA/1.

EXTRACTION OF *Cassia fistula* SEEDS

Seeds were collected and washed under running tap water and dried in oven at 50°C. The dried seeds were grinded to fine powder and stored in airtight bottles. 50gm of dried powder was defatted by treating with petroleum ether for 1-2 hour. Defatted powder was then packed in separating funnel extracted with 250 ml of 50% methanol (v/v). After 24 hr. solution was collected in a beaker and this process was repeated until transparent solution was appeared. The extract was filtered using whatman filter paper (No.1) and then concentrated in a vacuum and dried at 45-50 °C in Water bath for elimination of Solvents. The extracts were collected in a sterile airtight bottle under refrigerator of about 2-8°C for further use.

Animals:

The study was conducted on random bred, 6-7 weeks old and 24- 28 gm body weight bearing, male and female *Swiss albino* mice (*Mus musculus*). Animals were maintained under controlled conditions of temperature and light (Light: dark, 10 hrs: 14 hrs.). They were provided standard mice feed (procured from Hindustan Levers Ltd. India) and water *ad libitum*. The study protocol is approved by the Departmental Animal Ethical Committee and confirms to the guidelines set by World Health Organization, Geneva, Switzerland and Indian National Science Academy (INSA), New Delhi (India) (Project no 1695/PO/C/B/CPCSEA/1).

Chemicals: The Cyclophosphamide was purchased from Sigma chemical Co. U.S.A. and other chemical were reagents grade and purchased locally. All of the reagents were prepared in double distilled water to eliminate the contamination of metal ions. UV spectra were recorded in Shimadzu 1601 UV-Visible spectrophotometer.

Micronucleus Assay:
For the micronucleus assay, the extract at the volume of 0.2 ml at different doses level such as 500, 1000 and 1500 mg/kg body weight was injected 24 hours before the treatment of Cyclophosphamide, to six animals. The positive control group received single ip. injection of 50 mg/kg Cyclophosphamide in 0.9% saline the animals were sacrificed by cervical dislocation and bone marrow cells were harvested. The slides were prepared essentially as described by Schmid (1975) [14] and preston et al[15] and standardised by Agrawal et al [16] After staining with May-Gruenwald and Giemsa, a total 1000 cells were scored at the magnification of x1000 (100 x 10x) for each group. The data are expressed as the average number of micro nucleated cells/thousand polychromatied erythrocytes cells (PCE) cells/animals (±SE) for a group of six animals. The results were compared with the vehicle control group using Student's't' test with significance determined at $p<0.05$.

Analysis of slides

- 2000 polychromatic erythrocytes (PCEs) & nonchromatic Erythrocytes (NCEs) were scored for the presence of micronuclei at 10×100X magnification.
- Only PCE micronuclei were scored. The data was presented in MNPCE ± SE and PCE/NCE ± SE. The statistical significance was evaluated using Student's `t' test.

Study parameter

- Micronucleus count (Agrawal & Kumar 1998).
- PCE/ NCE ratio

Antioxidant Assay
Hydroxyl radicals were generated by a Fenton reaction system and the scavenging capacity towards the hydroxyl radicals was measured by using a deoxyribose method (Halliwell *et al.* 1987). The reaction mixture contained ferric chloride (Fe 1 mM 50µl), deoxyribose (3.6 mM, 100µl), ascorbic acid (1 mM 100 µl), EDTA (1 mM 50 µl), stock solution of hydromethanolic extracts at 10 mg / 20 ml were prepared from which 100 µl were added in reaction mixture, final volume was made up to 1ml by adding adequate quantity of phosphate buffer saline (pH, 7.4) and incubated for 1 hour at 37°C. The hydroxyl radical attached to deoxyribose and initiated a series of reaction that finally resulted in the formation of thiobarbituric acid reaction substance (TBARS). At last 0.5 ml of 5% TCA and 0.5 ml of 1% TBA was added. Mixture was than incubated for 20 minutes in a boiling water bath. The measurement of TBARS gives an index of free radical scavenging activity.

RESULTS
In the present study CP has been used as a clastogen and anticlastogenic effect of *C. fistula* has been observed in mice bone marrow cells (Table 1 & Graph 1 & 2 . Fig. 1). A significant reduced number of micronuclei were found in CF along with CP as compared the CP alone. The result of Micronucleus assay showed that single application of *Cassia fistula* Hydromethanolic seed extract (i.p.) at the dose of 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weight, 24 hours prior to the single i.p. administration of Cyclophosphamide (50 mg/kg) showed the reduction of micronucleus formation in PCE cell of bone marrow. The significant reduction of micronucleus formations was observed only in dose of 1500 mg/kg body weight, however 500 mg/kg, 1000 mg/kg has not shown much significant reduction in micronucleus formation in bone marrow cells of mice when compared to

Cyclophosphamide. The PCE/NCE ratio was not significantly affected in CF treated group as compared to carcinogen control which showed no toxicity of the extract in bone marrow cells of mice. While CP dose of 50mg/kg body wt. caused bone marrow toxicity as evidenced by a decrease in the proportion of PCE/NCE ratio.

The 50% methanolic extract of *C. fistula* exhibited a comparable antioxidant activity in Fenton reaction system. There was a dose dependent increase in the % TBARS formation for all concentrations tested (Table2 & Graph 3).The extract at a concentration of 100µg/ ml showed a percentage inhibition of 50.13 ± 1.45. IC 50 value of *C. fistula* extracts was found to be 100µg/ml (Table 3).

DISCUSSION

The treatment of human diseases has been attempted with varying degrees of success with the use of herbs. In present Study the antimutagenic potential of extract of *Cassia fistula* has been evaluated. The antimutagenecity of CF extract probably due to the presence of flavonoids. We found that CF extract showed anticlastogenic activity and was able to inhibit the formation of micronucleus induced by CY. Cyclophosphamide causes various types of oxidative DNA lesions by generating reactive oxygen species (ROS) (Sulkoawsk *et. al*.2011)[17.] ROS have been shown to cause gene mutation, DNA damage, chromosome aberrations and micronuclei formation. In present study seeds of CF extract decreased the formation of hydroxyl radicals and reduced the oxidative degradation of deoxyribose. So it can be presume that it may be free radical scavenging activity of CF which is responsible for the anticlastogenic effect of the extract against CP. It may be that micronuclei induced by CP may be due to the generation of free radicals, so antioxidative activities of CF could be modulate CP-induced genotoxicity. The present work suggests anticlasogenic and antioxidant activity of *C. fistula.*

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Table 1: Effect of *Cassia fistula* (CF) seed extract on Micronucleus (MN) formation induced by Cyclophosphamide (CP) in bone marrow cells of Swiss Albino mice.

S.no.	Groups	Treatment	MNPCE ± SEM	PCE/NCE ± SEM
1	I	Vehicle alone (0.2 ml D.W.)	0.12 ± 0.04	0.43 ± 0.08
2	II	CP alone (50 mg/kg)	4.3 ± 1.02	0.32 ± 0.122
3	III	*Cassia fistula* extract alone (500 mg/kg)	0.8 ± 0.1	0.83 ± 0.04
4	IV	*Cassia fistula* extract (500 mg/kg)) + CP (50 mg/kg)	4.5 ± 2.3	0.93 ±0.64
5	V	*Cassia fistula* extract (1000 mg/kg)+ CP (50 mg/kg)	4.0 ± 2.6	1.23 ± 0.64
6	VI	*Cassia fistula* extract (1500 mg/kg)+ CP (50 mg/kg)	0.6 ± 0.03*	1.91 ± 0.94

*denotes the level of significance as compared to positive control (Cyclophosphamide) group at $p < 0.05$ in Student 't' test.

Each group comprises of 6 animals.

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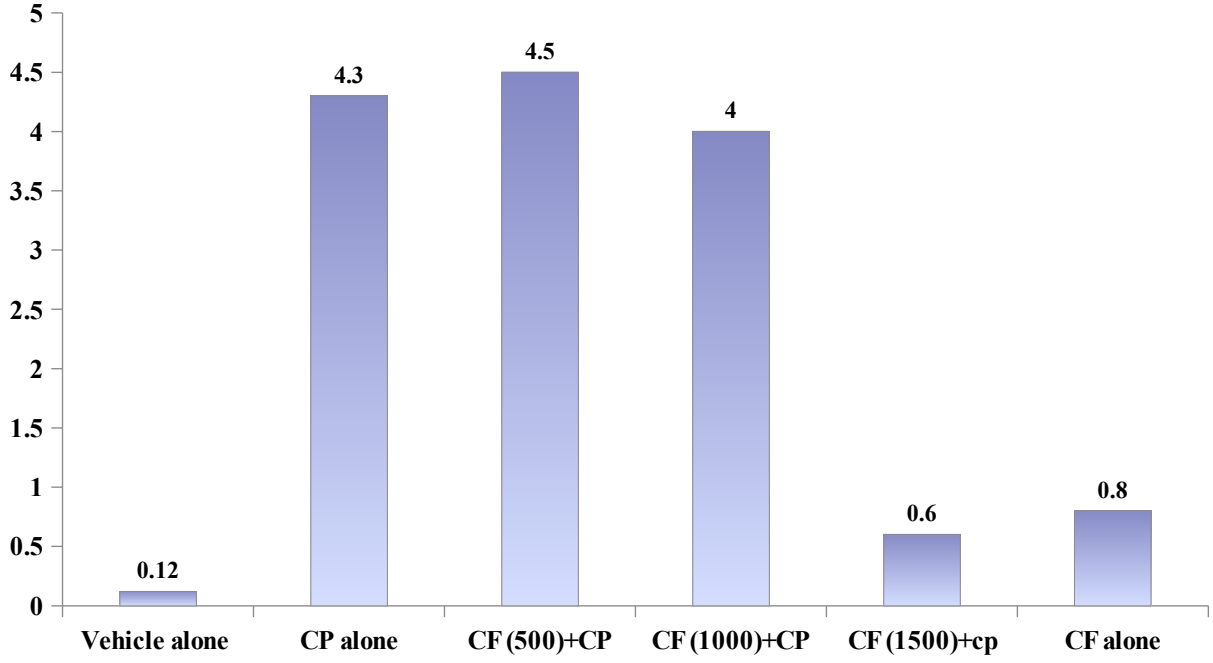
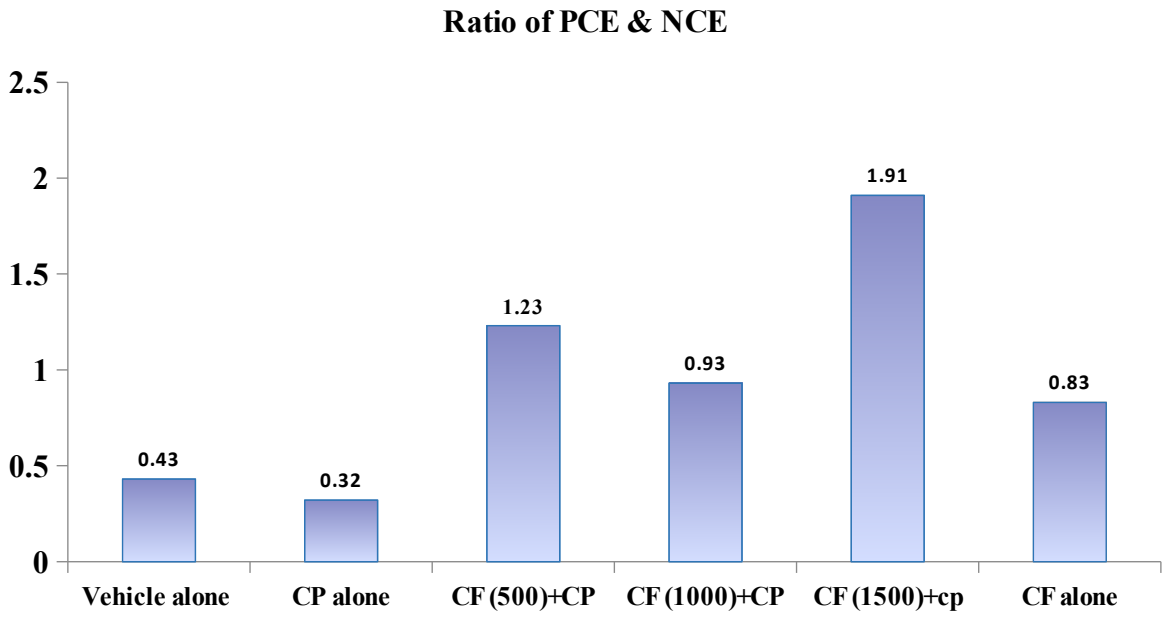


Graph 1: **Showing the effect of *Cassia fistula* (CF) seed extract on Micronucleus (MN) formation in bone marrow cells of Swiss Albino mice.**



Graph 2: Showing the effect of Cassia *fistula* (CF) seed extract on PCE: NCE ratio in bone marrow cells of Swiss Albino mice.

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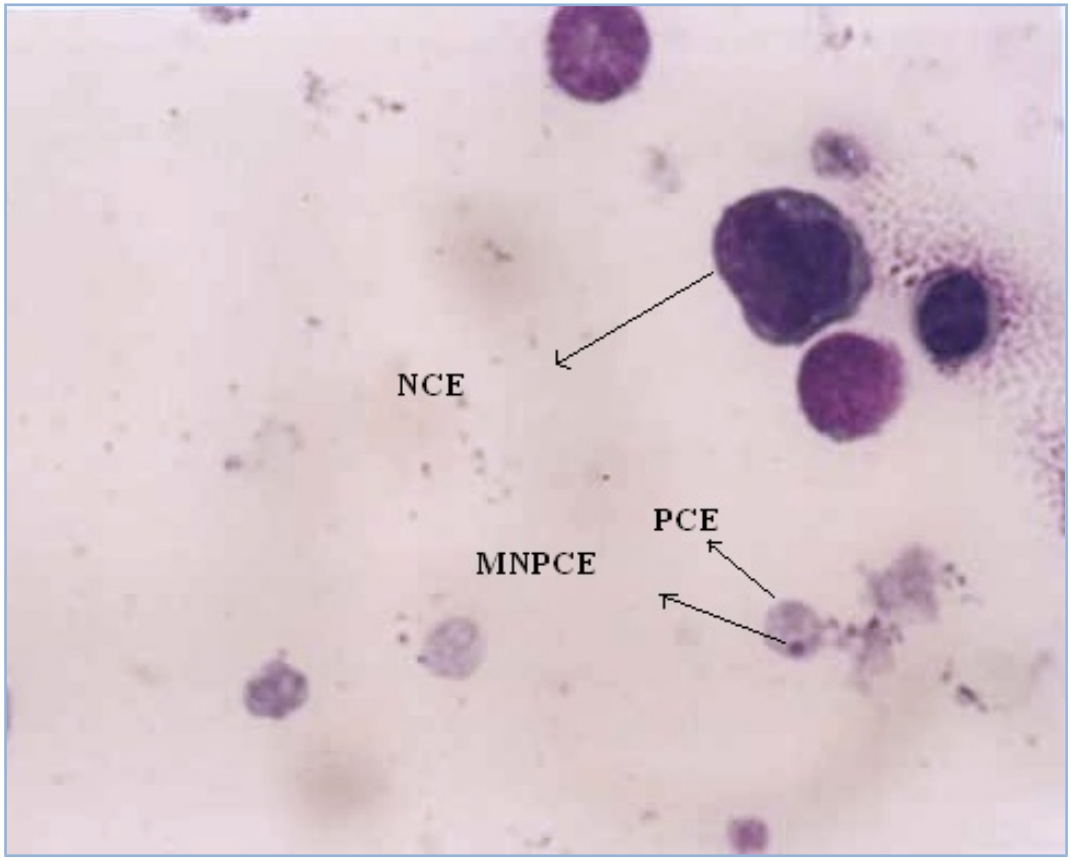

Figure 1: Showing MNPCE (Micro nucleated PCE) & NCE cells

Table 2 Antioxidant activity of Cassia fistula seed extract

S. No.	Concentration (µg/ml)	Ascorbic acid		CF extract	
		O.D. (532 nm)	% TBARS	O.D. (532 nm)	% TBARS
1	10	0.209	57.89	0.130	36.01
2	20	0.153	42.38	0.139	38.50
3	30	0.131	36.28	0.136	37.67
4	40	0.134	37.11	0.141	39.05
5	50	0.150	41.55	0.127	35.18
6	60	0.182	50.41	0.130	36.01
7	70	0.214	59.27	0.144	39.88
8	80	0.218	60.38	0.139	38.50
9	90	0.209	57.89	0.133	36.84
10	100	0.190	52.63	0.181	50.13

Table 3: IC50 value of *Cassia fistula* (CF) seed extract & Ascorbic acid as standard.

S. No.	Group	IC 50 Value
1	Ascorbic acid	60 (µg/ml)
2	*Cassia fistula*	100 (µg/ml)

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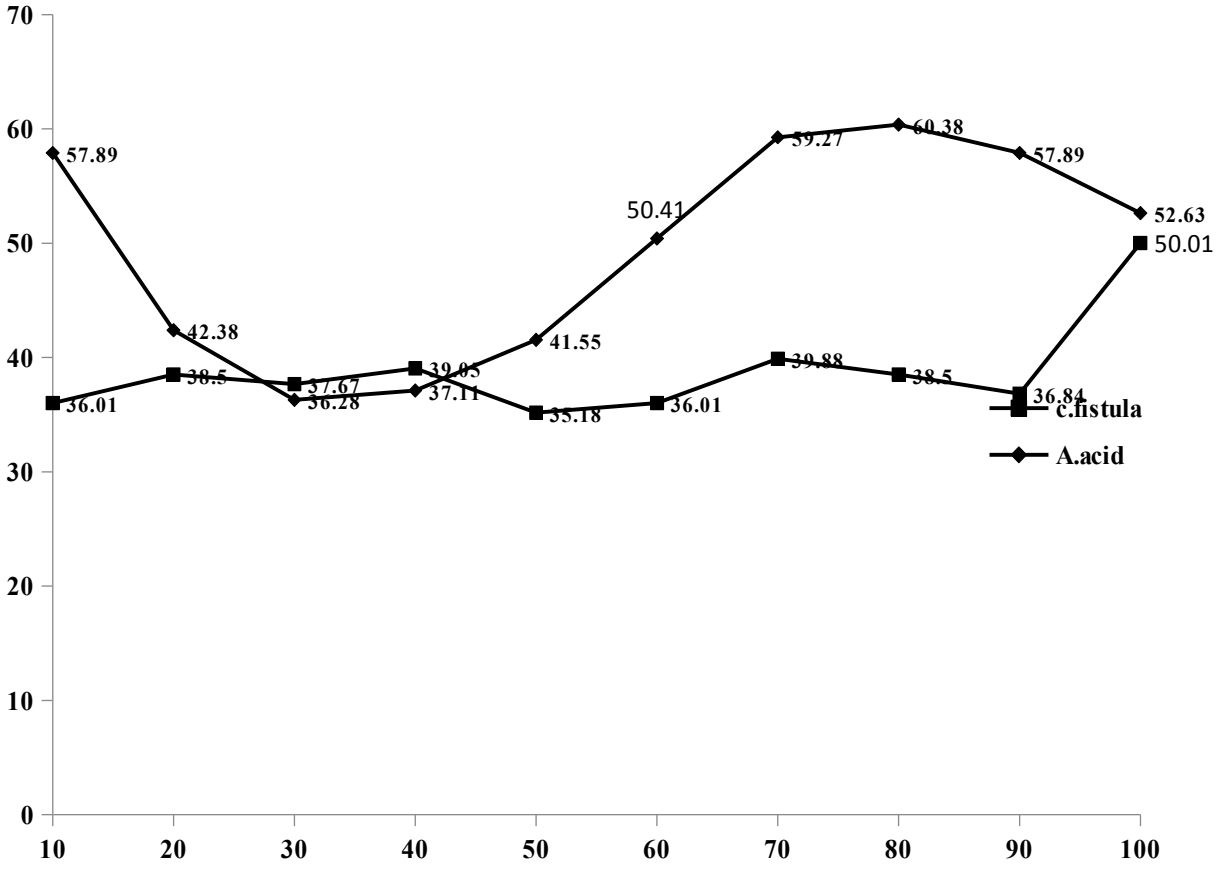

Graph 3: Showing the IC 50 value of CF and Ascorbic acid.